

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
11 January 2001 (11.01.2001)

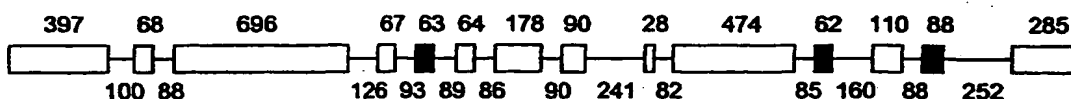
PCT

(10) International Publication Number  
**WO 01/02430 A3**

- (51) International Patent Classification<sup>7</sup>: C12N 15/54, 15/82, 15/29, 9/12, A01H 1/00
- (74) Agent: WITTOP KONING, T., H.; Exter Polak & Char-  
louis B.V., P.O. Box 3241, NL-2280 GE Rijswijk (NL).
- (21) International Application Number: PCT/EP00/06401
- (22) International Filing Date: 5 July 2000 (05.07.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 99202214.5 5 July 1999 (05.07.1999) EP
- (71) Applicants (for all designated States except US):  
CROPDESIGN N.V. [BE/BE]; Technologiepark 3,  
B-9052 Gent (BE). UNIVERSIDADE FEDERAL DO  
RIO DE JANEIRO [BR/BR]; CEP-21941-590 Rio de  
Janeiro, RJ (BR).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): HEMERLY, Adri-  
ana, Silva [BR/BR]; Rua Padre Achotegui, 60-1004,  
Leblon, CEP-22430-090 Rio de Janeiro, RJ (BR). FER-  
REIRA, Paulo, Cavalcanti, Gomes [BR/BR]; Rua Padre  
Achotegui, 60/1004, Leblon, CEP-22430-090 Rio de  
Janeiro, RJ (BR). ROMBAUTS, Stephane [BE/BE];  
Nederpolder 23, B-9000 Gent (BE).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,  
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,  
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,  
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,  
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,  
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian  
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European  
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,  
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,  
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:  
— with international search report
- (88) Date of publication of the international search report:  
27 September 2001
- For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: ARABIDOPSIS THALIANA CDC7 AND CDC27 HOMOLOGS

**CDC7 Gene Structure**



(57) Abstract: The present invention relates to at least partially purified protein, capable of modulating the DNA replication in plants, mutants thereof, DNA coding therefor and to a method to confer to one or more plant cells the capacity to provide such a protein or mutant. The invention also relates to plants, comprising the said DNA and the progeny thereof.

WO 01/02430 A3

## INTERNATIONAL SEARCH REPORT

Intr 'ional Application No

PCT/EP 00/06401

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/54 C12N15/82 C12N15/29 C12N9/12 A01H1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BEVAN, M. ET AL.: "Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of Arabidopsis thaliana"	14-16
Y	NATURE, vol. 391, no. 6666, 29 January 1998 (1998-01-29), pages 485-488, XP002157232 abstract  SEQ ID NO: Z97342 and gene no: 4515c page 486; figure 1; table 3 page 487, column 1, line 46 - line 53 -& EMBL database, Heidelberg, FRG Empln accession number Z97342 04 July 1997 BEVAN, M. ET AL.: "Arabidopsis thaliana DNA chromosome 4, ESSA I FCA contig fragment No. 7"	1-4, 6-13, 17-26

-/--

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"G" document member of the same patent family

Date of the actual completion of the international search

15 January 2001

Date of mailing of the international search report

18 APR 2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Fuchs, U

## INTERNATIONAL SEARCH REPORT

International Application No

PCI/EP 00/06401

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	<p>XP002157234 cited in the application the whole document -&amp; EMBL database, Heidelberg, FRG Trembl accession number 023540 01 January 1998 BEVAN, M. ET AL.: "PROTEIN KINASE HOMOLOG (KINASE LIKE PROTEIN) (Arabidopsis thaliana)" XP002157235 the whole document</p> <p>---</p>	
Y	<p>MASAI, H. ET AL.: "hsk1+, a Schizosaccharomyces pombe gene related to Saccharomyces cerevisiae CDC7, is required for chromosomal replication" EMBO JOURNAL, vol. 14, no. 13, 3 July 1995 (1995-07-03), pages 3094-3104, XP000978701 see especially page 3096; figure 1 the whole document</p> <p>---</p>	<p>1-4, 6-13, 17-26</p>
X	<p>WO 96 31608 A (JOHN INNES CENTRE INNOVATIONS LIMITED) 10 October 1996 (1996-10-10) abstract page 8, line 12 -page 9, line 1 page 10, line 1 - line 20 SEQ ID NOS: 1, 2 and 5 page 12, line 26 -page 21, line 20 page 39 -page 42; claims 3,8-11,13-18 page 45 -page 4; figure 1</p> <p>---</p>	<p>8,11-13, 15,16, 22,23</p>
X	<p>MIZOGUCHI, T. ET AL.: "ATMPKs: a gene family of plant MAP kinases in Arabidopsis thaliana" FEBS LETTERS, vol. 336, no. 3, 28 December 1993 (1993-12-28), pages 440-444, XP002079537 abstract page 441, column 1, line 21 -page 442, column 1, line 9 sequence of ATMPK4 page 441; figure 1</p> <p>---</p> <p style="text-align: center;">-/--</p>	<p>8,11,12, 15</p>

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/06401

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OHTOSHI, A. ET AL.: "Analyses of Saccharomyces cerevisiae Cdc7 kinase point mutants: dominant-negative inhibition of DNA replication on overexpression of kinase-negative Cdc7 proteins" MOLECULAR AND GENERAL GENETICS, vol. 254, no. 5, 20 May 1997 (1997-05-20), pages 562-570, XP002157233 cited in the application abstract	21
A	page 562, column 2, line 1 -page 563, column 2, line 55 page 564, column 1, line 11 -page 569, column 2, line 59; figures 1-4; tables 1-3 ---	1-4, 6-20, 22-26
A	HAMER, M.J. ET AL.: "Control of initiation of DNA synthesis in plants" PROCEEDINGS OF THE PHYTOCHEMICAL SOCIETY OF EUROPE, vol. 39, 1996, pages 211-224, XP000926262 page 211, line 1 -page 212, line 20 page 212, line 33 - line 34 page 213, line 32 -page 215, line 11; figure 16.2 page 216, line 37 -page 222, line 16 page 222, line 34 -page 223, line 6 ---	1-4,6-26
A	WO 98 41642 A (VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR BIOTECHNOLOGIE) 24 September 1998 (1998-09-24) the whole document ---	1-4,6-26
A	HEMERLY, A. ET AL.: "Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development" EMBO JOURNAL, vol. 14, no. 16, 15 August 1995 (1995-08-15), pages 3925-3936, XP002045514 the whole document -----	1-4,6-26

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/EP 00/06401

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims: it is covered by claims Nos.:  
1-3,6-26 partially and 4 completely

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## 1. Claims: 1-3, 6-26 partially and 4 completely

At least partially purified protein capable of modulating DNA replication in plants, at least comprising a) one or more of the amino acid sequences of SEQ ID NOS: 2, 3 and 4, b) one or more amino acid sequences having at least 50% amino acid identity with those of a), said protein comprising one or more of the amino acid sequences according to b) having an amino acid identity of at least 90%, said protein having the amino acid sequence as given in SEQ ID NO: 1 or having at least 50% amino acid identity with said sequence, said protein being a plant CDC7 protein or a functional analogue thereof, mutein of said protein, peptide comprising a) one or more of the amino acid sequences of SEQ ID NOS: 2, 3 and 4, b) one or more amino acid sequences having at least 50% amino acid identity with those of a), antibody specifically recognizing said protein, said mutein or said peptide, non-genomic DNA sequence coding for said protein, said mutein or said peptide or DNA sequence having a sequence homology of at least 75% of said sequence or complementary DNA sequence thereof, said DNA sequence comprising the DNA sequence as given by SEQ ID NO: 8 or having a sequence homology with said sequence of at least 75% or complementary sequence thereof, DNA sequence coding for said peptide corresponding to nucleotides 1229-1291, 2126-2187 or 2298-2385 of SEQ ID NO: 8 or a DNA sequence having a sequence homology of at least 75% to said sequence or complementary sequence thereof, DNA vector comprising said DNA sequence, method for modulating DNA replication in plant cells, plant parts or plants by conferring the capacity to provide said protein or said mutein in an amount sufficient to modulate DNA replication and/or block mitosis of said cells, method for identifying and/or obtaining proteins capable of modulating the DNA replication in plants comprising a two-hybrid screening assay using CDC7 polynucleotide sequences as bait and a cDNA library of a cell suspension culture as prey, method for the production of transgenic plants, plant cells or plant tissue comprising the introduction of said nucleic acid sequence or said vector into the genome of said plant, plant cell or plant tissue, plant cell transformed with said vector or comprising said DNA sequence, plant obtained by said method;

## 2. Claims: 1-3, 6-26 partially and 5 completely

At least partially purified protein capable of modulating DNA replication in plants, at least comprising a) one or more of the amino acid sequences of SEQ ID NOS: 6, 7, 10 and 12, b) one or more amino acid sequences having at least 50% amino acid identity with those of a), said protein comprising one or more of the amino acid sequences according to b) having an amino acid identity of at least 90%, said protein having the amino acid sequence as given in SEQ ID NOS: 5, 11 or 13 or having at least 50% amino acid identity

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

with said sequences, said protein being a plant CDC27 protein or a functional analogue thereof, mutein of said protein, peptide comprising a) one or more of the amino acid sequences of SEQ ID NOS: 6, 7, 10 and 12, b) one or more amino acid sequences having at least 50% amino acid identity with those of a), antibody specifically recognizing said protein, said mutein or said peptide, non-genomic DNA sequence coding for said protein, said mutein or said peptide or DNA sequence having a sequence homology of at least 75% of said sequence or complementary DNA sequence thereof, said DNA sequence comprising the DNA sequence as given by SEQ ID NOS: 9, 14 or 15 or having a sequence homology with said sequences of at least 75% or complementary sequence thereof, DNA sequence coding for said peptide corresponding to nucleotides 109-181, 2125-2181 or 1029-1061 of SEQ ID NO: 9, to nucleotides 109-181 or 2092-2148 of SEQ ID NO: 14 or to nucleotides 1-483 of SEQ ID NO: 15 or a DNA sequence having a sequence homology of at least 75% to said sequences or complementary sequence thereof, DNA vector comprising said DNA sequence, method for modulating DNA replication in plant cells, plant parts or plants by conferring the capacity to provide said protein or said mutein in an amount sufficient to modulate DNA replication and/or block mitosis of said cells, method for identifying and/or obtaining proteins capable of modulating the DNA replication in plants comprising a two-hybrid screening assay using CDC27 polynucleotide sequences as bait and a cDNA library of a cell suspension culture as prey, method for the production of transgenic plants, plant cells or plant tissue comprising the introduction of said nucleic acid sequence or said vector into the genome of said plant, plant cell or plant tissue, plant cell transformed with said vector or comprising said DNA sequence, plant obtained by said method;

# INTERNATIONAL SEARCH REPORT

International Application No

PC1/EP 00/06401

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9631608 A	10-10-1996	AU 703644 B	25-03-1999
		AU 2415495 A	05-12-1995
		AU 703525 B	25-03-1999
		AU 5282396 A	23-10-1996
		CA 2188562 A	23-11-1995
		CA 2216406 A	10-10-1996
		CN 1190439 A	12-08-1998
		EP 0759086 A	26-02-1997
		EP 0819174 A	21-01-1998
		WO 9531564 A	23-11-1995
		JP 10500010 T	06-01-1998
		JP 11503319 T	26-03-1999
-----			
WO 9841642 A	24-09-1998	AU 6730198 A	12-10-1998
		EP 0972060 A	19-01-2000
-----			